Eur Food Res Technol (2015) 240:1023–1034
DOI 10.1007/s00217-014-2406-6

ORIGINAL PAPER

Biosynthesis of β(1,3)/(1,6)-glucans of cell wall of the yeast *Candida utilis* ATCC 9950 strains in the culture media supplemented with deproteinated potato juice water and glycerol

Anna Bzducha-Wróbel · Stanisław Błażejak · Magdalena Molenda · Lidia Reczek

Received: 21 September 2014 / Revised: 24 November 2014 / Accepted: 26 November 2014 / Published online: 5 December 2014

Abstract Yeast cell wall β-glucans exhibit valuable functional properties, including therapeutic ones. The possibility of using deproteinated potato juice water (DPJW) with the addition of glycerol in yeast culture of *Candida utilis* ATCC 9950 for biosynthesis of β(1,3)/(1,6)-glucans of cell wall was determined. The biosynthesis of β-glucans was preferably intensified by media prepared from DPJW at pH 5.0 with 5 or 10 % addition of glycerol. Cells from these cultures were characterized and found to possess thickest cell walls and β-glucan layers. The content of β-glucans in the walls' preparations was estimated to be approximately 44–45 % and was significantly higher in comparison with the content after culturing in synthetic YPD medium (approximately 31 %). The new developed media may be used in the production of *C. utilis* biomass with enhanced content of functional β-glucans. It creates the possibility of a favorable, simultaneous management of waste potato juice water and glycerol in the formation of β(1,3)/(1,6)-glucans.

Keywords β(1,3)/(1,6)-Glucan · Deproteinated potato juice water · Glycerol · *Candida utilis* · Cell wall

A. Bzducha-Wróbel (✉) · S. Błażejak · M. Molenda
Department of Biotechnology, Microbiology and Food Evaluation, Faculty of Food Science, Warsaw University of Life Sciences-SGGW, Nowoursynowska Str. 159c, 02-776 Warsaw, Poland
e-mail: anna_bzducha_wrobel@sggw.pl

L. Reczek
Department of Civil Engineering, Faculty of Civil and Environmental Engineering, Warsaw University of Life Sciences-SGGW, Nowoursynowska Str. 159c, 02-776 Warsaw, Poland

Introduction

Beta(1,3)/(1,6)-glucans are structural polysaccharides of the yeast cell wall. They exhibit diverse functional properties, including therapeutic, thanks to which they find an application in the pharmaceutical, cosmetic, food, and other industries [23]. They serve as biological modifiers of the immune response. Additionally, they stimulate antimicrobial and anti-inflammatory activities; adsorb mycotoxins; decrease the fraction of LDL (low-density lipoprotein) cholesterol; exhibit anticancer, antimutagenic, and antioxidant properties; and promote wound healing [4, 7, 18, 25, 40].

They can act as emulsifiers, gelling agents, or water- and fat-binding substitutes of fat, prebiotics, and film-forming substances [23, 31]. Since 2011, β(1,3)/(1,6)-glucans extracted from the yeast *Saccharomyces cerevisiae* have been used in the EU countries in certain food categories, as components of the functional foods and of particular nutritional uses [8]. It opens the possibility for searching new, efficient sources of these polymers. It is reasonable especially because the content of structural components of the cell wall and their chemical structure vary considerably depending on the species and strain of yeast, culture conditions (type and amount of carbon and nitrogen sources, type and availability of minerals, pH, temperature, culture period, and degree of culture aeration), and age of cells [1, 3, 11, 14, 15, 17, 30, 45].

The influence of the above-mentioned factors on the biosynthesis and the construction of structural polymers of the yeast cell wall have not been described precisely in the literature.

Parameters such as molecular structure, degree of polymerization, molecular branching, molecular weight, and polysaccharides solubility of the yeast wall determine the functional properties of these polymers [4].

Production of β(1,3)/(1,6)-glucans is based on their isolation from yeast biomass. From the biotechnological viewpoint, efficiency of the process can be increased by stimulating biosynthesis of these polysaccharides and/or gaining higher productivity of the yeast biomass. This can be achieved by an appropriate selection of culture medium composition and growth conditions as well. Optimization of the culture medium composition used in the biosynthesis processes of the desired metabolite is one of the most important aspects of industrial biotechnology. The costs of the whole biotechnological process are determined primarily by the cost of the medium [33]. Industrial wastes, for which new directions of management have constantly been searching for, are deproteinated potato juice water (also called potato juice water) and glycerol. They may constitute a source of digestible nutrients for microorganisms.

Deproteinated potato juice water is a by-product formed during the production of potato starch after acid-thermal coagulation of proteins from potato juice. During the processing of 1,000 tons of potatoes, approximately 600 m^3 of deproteinated potato juice water is produced [22]. After dilution by industrial water, this effluent is used as a reservoir fertilizing the soil. Because of the high levels of indicators such as biological oxygen demand during 5 days (BOD_5, approximately 4,800–2,600 mg O_2/L) and chemical oxygen demand (COD, approximately 7,700 mg O_2/L), the utilization of the above-mentioned effluent as a fertilizer significantly burdens the environment [21, 22]. Its use as a component of media for microorganisms culturing in the process of obtaining valuable products from their metabolism may become one of the most effective methods of deproteinated potato juice water management. So far, attempts have not been sufficiently described in the literature [29].

Glycerol is the main by-product generated in the production of biodiesel. The increase in biofuel production generates increasing amounts of glycerol fraction and is associated with this need of searching alternative methods of its disposal [9].

The objective of the study was to determine the effect of *Candida utilis* ATCC 9950 fodder yeast culturing, carried out in media with deproteinated potato juice water with the addition of glycerol, on the structure of cell wall in the context of the possibility of obtaining yeast biomass with increased content of β(1,3)/(1,6)-glucans. It was assumed that the use of deproteinated potato juice water as a source of nitrogen and minerals and glycerol as a carbon source may form the content of *Candida* cell wall components. *C. utilis* yeasts, selected in the preliminary study, as a potential source of β-glucans, have a long history of safe use, which is important for the possibility of industrial use of microbial-derived substances. Previous studies confirm that structural polymers of cell wall of this yeast species exhibit desirable biological and technological properties [19, 44].

Materials and methods

Microorganism

The studies were conducted with the use of *C. utilis* ATCC 9950 fodder yeast from the collection of pure cultures of the Department of Biotechnology and Microbiology, Warsaw University of Life Sciences. Biological material was stored on YPD slants at a temperature of 6–8 °C.

Preparation of deproteinated potato juice water

Deproteinated potato juice water was prepared on a laboratory scale from potatoes of Irga variety (120 kg) bought in July 2013. Procedure for obtaining deproteinated potato juice water was similar to that used in factories producing potato starch. Potatoes were mechanically crumbled, as a result of which cell juice was secreted followed by its separation from potato pulp. Subsequently, the starch was separated from the juice by centrifugation without temperature control (3,200*g*/20 min, Eppendorf 5810 Centrifuge). The next step was the acid-thermal coagulation of proteins present in the potato juice [35]. Then, pH of the juice was set at an approximate level of 5.0 using concentrated sulfuric acid, followed by sterilization process (121 °C/0.1 MPa/20 min, HICLAVE HG-80 autoclave, HMC Europe). Coagulated protein was separated by filtration. The filtrate, namely deproteinated potato juice water, was used to prepare the experimental media.

Chemical characteristics of deproteinated potato juice water

The prepared deproteinated potato juice water was characterized in terms of COD (Hach micromethod, Hach Lange analyzer) and BOD_5 using OxiTop Control Lovibond bottles. The results of both measurements were expressed in g O_2/L of deproteinated potato juice water.

To define deproteinated potato juice water as a culture medium, total organic carbon (TOC) was determined using high-temperature oxidation method using IL 550 TOC-TN analyzer. Results were expressed in grams of carbon per liter of culture medium (g C/L). Dry matter was determined by drying-weighing method (105 °C/24). Total nitrogen was determined using Kjeldahl method (BÜCHI mineralization and distillation units). To calculate nitrogen per protein, a ratio of 6.25 was used. Directly reducing sugars and total sugars (after previous acid hydrolysis of samples) were determined by colorimetric method with 3,5-dinitrosalicylic acid. The results of these above-mentioned measurements were expressed in grams per liter of medium.

The content of selected elements (P, K, Na, Ca, Mg, and S) in deproteinated potato juice water was determined

by ICP (inductively coupled plasma) technique in atomic emission spectrometer (ICP-AES, Thermo iCAP 6500 Duo at the wavelength proper for each element (Ca: 315.8, 373.6, and 422.6 nm; K: 766.4 and 769.8; Mg: 279.5, 280.2, 285.2, and 382.9; Na: 588.9 and 589.5; P: 177.4, 178.2, and 213.6; and S: 180.7 and 182.0). The samples of deproteinated potato juice water (1 cm^3) for the determination of chemical element contents were mineralized in a mixture of nitric acid and perchloric acid (Büchi Digestion Unit K-435). The results were stated in grams of element adjusted to a 1 L of the medium (g/L). Selected measurements were also conducted in synthetic YPD medium.

Culture media

Experiments were performed in 16 variants of experimental media. Control cultures of the yeast *C. utilis* ATCC 9950 strains were cultured in a synthetic yeast extract-peptone-glucose medium (YPD) composed of (g/L): 20 g peptone, 20 g glucose, and 10 g yeast extract. This is a complete yeast culture medium, which was treated as a reference medium. The components of YPD medium were dissolved in distilled water. The active acidity of the medium was established at pH 5.0.

Experimental media from deproteinated potato juice water were differentiated in terms of glycerol concentration as a carbon source. Doses of 50, 100, 150, 200, and 250 g of glycerol/L deproteinated potato juice water were investigated. At this stage of research, commercial glycerol of analytical grade (POCH, Poland) was used. Media with deproteinated potato juice water with glycerol were prepared in three different pHs (4.0, 5.0, and 7.0). All media were sterilized at a temperature of 121 °C (0.1 MPa) for 20 min (HICLAVE HG-80 autoclave, HMC Europe).

In the description of figures and tables containing the results, abbreviations of used experimental media were applied according to the rule: pH of the medium_glycerol concentration %.

Preparation of *inoculum*

Yeast *inoculum* was prepared in liquid YPD medium (80 cm^3) in a 500-cm^3 volumetric flask. The medium was inoculated with material collected from the slants. Cultures were grown at 28 °C for 20 h on a reciprocating shaker (SM-30 control) with a frequency of 200 cycles/min. After incubation, the yeast biomass was centrifuged (15,500*g*/4 °C/10 min, Eppendorf 5810R Centrifuge) and rinsed twice from the medium components with sterile water and suspended in 80 cm^3 of saline. Inoculum obtained by such method (approximately 7×10^7 cells/cm^3) constituted material for inoculation of microculture in Bioscreen C system and for proper culture experiments.

Screening of the growth of the studied yeast in experimental media with the use of Bioscreen C system

Growth of studied *C. utilis* ATCC 9950 in experimental media was evaluated in microcultures with the use of Bioscreen C system. About 250 µl media inoculated with 10 % volume of yeast inoculum (6.8×10^5 cell/cm^3) were added to each culture plate well. Cultures were grown at a temperature of 28 °C for 110 h with intensive shaking. Changes in optical density (OD) of the culture were monitored each hour using OD readout with a broadband filter (420–580 nm), insensitive to color changes of the media. Based on the obtained results, growth curves of the studied yeasts in experimental media were prepared, and the duration of the adaptive phase (t_{lag}), the minimum and the maximum OD values in the logarithmic phase growth ($OD_{min/max}$), and the duration of the logarithmic phase growth (Δt_{log}) were determined. Moreover, the maximum rate of yeast growth in logarithmic phase (μ_{max}) was calculated using the formula: $\mu_{max} = (\ln OD_{max} - \ln OD_{min})/\Delta t_{log}$ [h^{-1}] and the generation time ($g = \ln 2/\mu_{max}$ [h]).

Experimental culture conditions

Experimental cultures were grown in flasks of 500 cm^3 volume. Media (90 cm^3) were inoculated with *inoculum* material constituting 10 % of the culture volume. The cultures were incubated for 24 h at 28 °C on SM-30 Control (Buechler, Germany) reciprocating shaker at a frequency of 200 cycles/min. Each culture variant (in reference to the culture medium) was replicated three times.

Biomass yield

To determine the yeast biomass yield, 5 cm^3 of cultivation medium after 24-h incubation was centrifuged (15,500*g*/4 °C/10 min, Eppendorf 5810R Centrifuge). The centrifuged biomass was rinsed twice with deionized water and then dried at 105 °C to achieve a constant mass (approximately 24 h). The biomass yield was stated in grams of yeast dry substance adjusted to a 1 L of cultivation medium basis (grams of dry substance per liter of medium).

Analysis of yeast cell wall structure using transmission electron microscopy

The aim of this study phase was to determine the yeast cell wall total thickness, as well as the β-glucan and mannoprotein layer thickness in response to yeast cultivation conditions. For microscopic observations, yeast biomass specimens were collected after 24 h of cultivation. To prepare yeast biomass for microscopic observations, it was

necessary to fix yeast cells in a glutaraldehyde (2.5 %) prepared in phosphate buffer (pH = 7.2). Then, the specimens were fixed in an osmium solution (OsO_4). Further procedure phases were as follows: preparation rinsing with water, dehydration with ethanol, embedding in Epon resin, cutting sections by means of a ultramicrotome (LKB), and contrasting with uranyl acetate and lead acetate [5]. Measurements of yeast cell wall thickness, mannoprotein, and β-glucan layer thickness were performed on the grounds of photographs made with the transmission electron microscope (TEM; JEM-1220 type JOEL).

Approximately 100 images of cells from each experimental medium variant were taken. Measurements of the thickness of the cell wall and structural layers (β-glucan and mannoprotein) were performed in MultiScan v. 18.03 program in five different areas of the cell wall of studied yeasts in each analyzed image.

Cell wall preparations

To obtain preparations of yeast cell walls, disintegration of cells was performed. The cells were destroyed mechanically in a Bead-Beater homogenizer, model GB26 (Biospec Products), with a power of 400 W. The total working volume of the reservoir mill was approximately 300 cm^3. In the experiments, Zirconia-glass beads (Biospec Products) of 1 mm diameter were used. The beads were added at a volume of 170 cm^3, which accounted for 57 % of reservoir working capacity. About 100 cm^3 of yeast biomass suspension was subjected to homogenization in deionized water (Direct-Q 3UV-R, Millipore). The biomass was collected from three parallel culture flasks for each variant of experimental medium. Throughout the duration of the disintegration process, the reservoir was cooled with ice bath jacket. The disintegration consisted of seven cycles: 3 min of working and 3-min interval for each. To recover disintegrated cell walls of yeasts, beads were washed three times with 50 cm^3 of deionized water. Disintegrated cell suspension was separated by centrifugation (5,000*g*/4 °C/5 min, Eppendorf 5810R Centrifuge). The effectiveness of cell disintegration and the degree of purification of wall preparations from cytosol components were evaluated microscopically. Preparations were prepared directly after the disintegration of the cells followed by staining with methylene blue. Microscopic observations were carried out under MB300 (OPTA-TECH) light microscope at 600-fold magnification. Preparations' images were taken with the use of a camera and OptaView 7 (OPTA-TECH) computer program.

Before further analyses, precipitates of cell wall preparations obtained after the disintegration of the cells were subjected to preliminary treatment from the components of cytosol. To this end, 30 cm^3 of deionized water was added to the pellet obtained as a result of centrifugation. Subsequently, the content of samples was vortexed followed by centrifugation (5,000*g*/4 °C/5 min, Eppendorf 5810R Centrifuge), and the supernatant was decanted. The precipitate was rinsed three times with 30 cm^3 of NaCl solution (POCH) with increasing concentrations, that is, 17, 34, and 85 mM. The last two washings of the precipitate were carried out using deionized water. Each time the samples were centrifuged under the above-mentioned parameters of centrifugation. The preparations obtained in such way were dried at 80 °C for 24 h followed by crushing in MKM 6003 (BOSH) mill.

Characteristics of cell wall preparations

Determination of total saccharides content

The content of total saccharides as reducing sugars expressed per glucose was determined with the colorimetric method with dinitrosalicylic acid (DNS) at the wavelength of 540 nm (BIO-RAD SmartSpec 3000) [43]. Before determination, cell wall polymers (approximately 20 mg) were subjected to acidic hydrolysis (72 % H_2SO_4, 95 °C/4 h) in water bath (Memmert WNB14, Germany) [10]. The temperature of hydrolysis was modified from 100 to 95 °C without influencing the process efficiency.

The content of saccharides was calculated using a standard curve prepared for glucose ($y = 2.7369x - 0.0594$, $R^2 = 0.9981$).

Enzymatic determination of β(1,3)(1,6)-glucan content

The total content of β(1,3)(1,6)-glucan was determined using an Enzymatic Yeast Beta-Glucan Kit by Megazyme (K-EBHLG 03/13) following procedure recommended by the producer [31]. The UV-1800 UV/VIS, RAYLEIGH spectrophotometer was used.

Statistical analysis of the results

The obtained results were subjected to a statistical analysis using the STATISTICA V.10 programme. An analysis with the ANOVA method (Tukey's test) was carried out at the $\alpha = 0.05$ level of significance.

Results and discussion

Characteristics of deproteinated potato juice water

The first stage of the conducted studies was to characterize deproteinated potato juice water in terms of its suitability for cultivation of microorganisms as a source of nutrients.

Table 1 Characterization of deproteinated potato juice water and its comparison with synthetic YPD medium

Parameter	Unit	Medium	
		Deproteinated potato juice water	YPD
COD	(g O_2/L medium)	30.0	–
BOD_5		15.8	–
Dry substance	($g_{d.s.}$/L medium)	37.6 ± 0.0	50.0 ± 0.0
Total nitrogen	(g/L medium)	2.6 ± 0.0	3.9 ± 0.0
Protein		16.0 ± 0.0	24.4 ± 0.0
Directly reducing sugars		7.0 ± 0.1	21.7 ± 0.2
Total sugars		7.9 ± 0.1	21.8 ± 0.2
Total organic carbon		14.7 ± 0.0	22.7 ± 0.0
Phosphorus		0.420 ± 0.021	0.291 ± 0.014
Potassium		3.930 ± 0.254	0.817 ± 0.063
Sodium		0.584 ± 0.058	1.223 ± 0.032
Calcium		0.563 ± 0.043	0.624 ± 0.060
Magnesium		0.310 ± 0.015	0.059 ± 0.010
Sulfur		5.883 ± 0.930	3.650 ± 0.883
C:N	–	5.64	5.82
C:N:P ratio		5.64:1:0.161	5.82:1:0.075

For this purpose, in deproteinated potato juice water, the content of dry substance, total organic carbon (TOC), total sugars, and directly reducing sugars, nitrogen, protein, and minerals such as phosphorus, potassium, sulfur, sodium, calcium, and magnesium were determined. Deproteinated potato juice water was also subjected to an analysis, which determines chemical oxygen demand (COD) and biochemical oxygen demand (BOD_5). The characteristics of deproteinated potato juice water were compared with synthetic YPD medium commonly used in yeast culture. The obtained results are listed in Table 1.

Deproteinated potato juice water is a difficult by-product to manage in the potato industry creating the environmental risk due to the high burden of organic and inorganic substances [33]. Indicators that evidence about a significant burden of sludge by deproteinated potato juice water are high values of COD, BOD_5, and the total content of phosphorus and nitrogen substances (Table 1). Based on Polish national Regulation of Minister of Environment [37], the acceptable COD, BOD_5, total nitrogen and phosphorus contents in liquid wastes loading into water or ground are as follows: 125 mg O_2/L, 25 mg O_2/L, 15 mg N/L, and 4 mg PO_4/L, respectively. It confirms the relevance of searching effective methods of management of the described industrial waste.

Important parameters characterizing culture medium are the ratio of carbon:nitrogen:phosphorus (C:N:P) and carbon:nitrogen (C:N) as well. Availability and mutual proportions of the above-mentioned elements are important from the viewpoint of microbial biomass cultivation and optimization of conditions for the biosynthesis of certain products of microbial metabolism [27, 36, 41]. In the production of yeast biomass, the most favorable ratio of carbon to nitrogen and phosphorus (C:N:P) stands at approximately 6.25:1:0.125 [27]. In deproteinated potato juice water, this ratio was 5.64:1:0161. The C:N ratio designated for deproteinated potato juice water (5.64) was comparable with that observed in synthetic YPD medium (5.82); however, deproteinated potato juice water contained approximately 35 % less carbon in total and three times less reducing sugars (Table 1), which are an easily absorbed source of this element for the microorganisms. Therefore, deproteinated potato juice water was supplemented with additional carbon source to preferably balance its availability in media from this waste. In the present study, glycerol was used for this purpose (Table 2).

The addition of glycerol to deproteinated potato juice water increased the content of TOC in the developed culture media within the range from approximately 36.6 g/L (at 5 % glycerol) to approximately 124.5 g/L (at 25 % glycerol; Table 2). In these media, C:N ratio was ranged from approximately 14 to about 48. However, it should be noted that various carbon sources may be subjected to different routes of transport into the cell interior and other metabolic processes and influence the increase in osmotic pressure to various extent. Hence, the use of only C/N ratio is not sufficient.

The results of the conducted measurements confirmed that deproteinated potato juice water constitutes a good source of nitrogen and minerals, which stimulate microbial growth (Table 1). The presence of protein in deproteinated potato juice water at the level of approximately 1.6 %

Table 2 Total carbon content and C/N ratio in media from deproteinated potato juice water depending on glycerol concentration

Deproteinated potato juice water supplemented with glycerol	TOC (g/L)	C/N ratio
Glycerol (%): 5	36.6	14.3
10	58.6	22.8
15	80.5	31.3
20	102.5	39.9
25	124.5	48.4

confirmed the possibility to use waste potato juice water as a cheap equivalent of nitrogen source, with respect to expensive components of the synthetic media used in yeast culture.

The studied medium proved to be a better source of phosphorus, magnesium, potassium, and sulfur as compared with YPD medium. The first two elements are particularly important for the yeast growth.

The synthesis process of microbial-derived β(1,3)-glucans may depend on the availability of phosphorus, sulfur, and cationic composition in the culture medium [11, 23]. In deproteinated potato juice water, a high sulfur content was reported. This result was certainly affected by potato acidification with sulfuric acid in the process of acid-thermal protein coagulation. In contrast, the content of this element in the potato juice depends on the availability of sulfur in the soil. Sulfur is accumulated, among others, in sulfur-containing amino acids that build the protein of potato [34]. Acid-thermal coagulation of proteins from potato juice contributes to precipitation of approximately 80 % proteins, while the low molecular weight fractions remain in the dissolved form [34]. Availability of sulfur-containing amino acids in the yeast growth medium may affect the synthesis of cell wall polysaccharides of these fungi [15].

Potato juice is also a source of vitamins B1, B2, B6, PP, C, and E [21], which positively affects the growth of microorganisms.

Nowak et al. [32] attempted to perform bioremediation of sludge from the potato industry including deproteinated potato juice water, with the use of mixed bacterial culture. The sludge used in the studies of above-cited authors came from potato-processing factories and was characterized by the total content of nitrogen and phosphorus (2.11 and 0.43 g/L) and COD and BOD_5 indicators (approximately 33 and 16.5 g O_2/L, respectively) comparable with those for deproteinated potato juice water obtained in the course of our studies at a slightly lower TOC values, the content of reducing sugars (approximately 9.4 and 3.4 g/L, respectively), and dry substance (approximately 31 $g_{d.s.}$/L). It should be noted that the composition of potato-processing sludge may vary, especially in the content of reducing substances, depending on whether they are produced in autumn or spring campaign [33].

Table 3 Parameters characterizing the growth of *C. utilis* ATCC 9950 in experimental media based on microculture in Bioscreen C system

Culture medium	pH	t_{lag} (h)	Δt_{log} (h)	μ_{max} (h^{-1})	Generation time (h)
YPD	5.0	4	15	0.117	5.94
DPJW + glycerol: 5 %	4.0	7	10	0.117	5.95
10 %		8	11	0.104	6.65
15 %		9	12	0.098	7.06
20 %		10	13	0.072	9.58
25 %		13	22	0.052	13.24
DPJW + glycerol: 5 %	5.0	6	10	0.117	5.92
10 %		6	10	0.115	6.03
15 %		7	10	0.106	6.57
20 %		8	11	0.075	9.19
25 %		10	20	0.051	13.69
DPJW + glycerol: 5 %	7.0	6	10	0.103	6.74
10 %		7	11	0.091	7.61
15 %		8	15	0.072	9.66
20 %		9	16	0.070	9.93
25 %		10	16	0.067	10.29

Growth screening of studied yeasts in the experimental media

For rapid evaluation of growth of the studied *C. utilis* ATCC 9950 strains, in the developed experimental media, the analysis of changes in OD of microculture was conducted in Bioscreen C system. The obtained results allowed to plot growth curves of the investigated media and to determine basic parameters of *C. utilis* ATCC 9950 growth such as the length of the adaptive and the logarithmic phase, the maximum growth rate, and the generation time of the cells. The results are listed in Table 3.

It was found that *C. utilis* ATCC 9950 exhibited growth in every variant of the experimental medium. It was reported that the most favorable culture conditions were observed at pH 5.0 with the addition of 5 and 10 % glycerol and in the medium at pH 4.0 containing 5 % glycerol. The maximum growth rate (0.117 and 0.115 1/h) of the studied yeast under these conditions was comparable with that observed in synthetic YPD medium (0.117 1/h). It was reflected in the shortest time of generation reported for cells in mentioned culture media (approximately 6 h). In media with deproteinated potato juice water and glycerol,

adaptive phase of cells was longer by approximately 2–3 h as compared with that of cells in YPD medium, while the logarithmic phase growth appeared to be shorter by 5 h.

Rapid and efficient growth of the studied yeasts was also observed in the media at pH 7.0 containing 5 % glycerol.

It was found that the factor, which determines the growth of the studied yeasts in the developed media, was the concentration of glycerol in medium. With the increase in the concentration of this carbon source in the medium, extension of adaptive phase and slowdown of growth rate of yeast cells and extension of their generation time were reported (Table 3). Higher concentrations of glycerol in medium constituted as a stress factor potentially associated with an increase in the osmotic pressure in media containing this low molecular weight carbon source. The plasma membrane of yeast cell has semipermeable properties with regard to water and higher molecular weight hydrophilic compounds. Glycerol is typically transported across the cell membrane via the glycerol channel (Fps1p) by facilitated diffusion over a concentration gradient. In hyper-osmotic environment, closing of Fps1p is required for intracellular accumulation of osmolytes that regulate the intracellular pressure (e.g., trehalose or intracellular glycerol) [2, 12]. Consequently, diffusion of glycerol (as a carbon source) from culture medium into yeast cells was restricted.

Further studies were conducted in all variants of the experimental media keeping in mind that stress conditions of yeast growth affect the content of structural polymers in the cell wall of yeast [17, 42].

Biomass yield

Biomass yield of yeasts in the developed media was determined after 24 h of culturing. The results are shown in Fig. 1. Based on these results, the relevance of *C. utilis* ATCC 9950 culturing was affirmed in experimental media, in which one achieved the highest amounts of biomass as a source of $\beta(1,3)/(1,6)$-glucans of the cell wall (DPJW at pH 5.0 and 4.0 with 5 and 10 % glycerol as well as DPJW at pH 7.0 containing 5 % of glycerol).

Observations reported in microcultures of Bioscreen C system were confirmed by results of yeast biomass yield obtained in studied culture mediums'. The highest yields of biomass were obtained in media prepared from deproteinated potato juice water at pH 5.0 and 4.0 with the addition of 5 and 10 % glycerol and in the medium of pH 7.0 containing 5 % of this carbon source. These were values of 11–12.7 $g_{d.s.}/dm^3$ order of magnitude, comparable with those achieved after culturing of the studied *C. utilis* ATCC 9950 fodder yeast in a synthetic YPD medium. Similar efficiency of yeast biomass in the described media with deproteinated potato juice water and glycerol, achieved in a shorter time as compared with that in YPD medium (Table 3), seems to

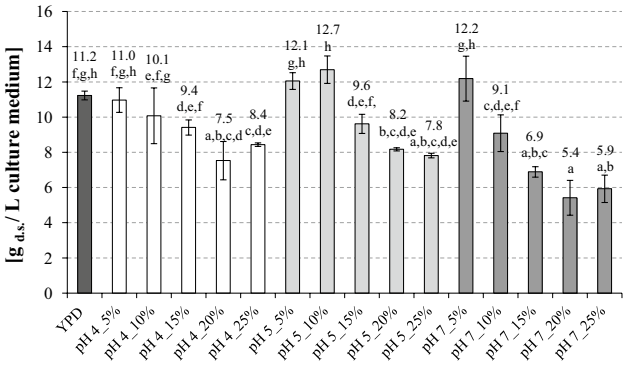

Fig. 1 Biomass yield of *C. utilis* ATCC 9950 from 24 h of experimental culture (*a*, *b*, *c* mean values marked with the *same letters* do not differ significantly, Tukey's test, $\alpha = 0.05$)

be interesting taking into account the possibility of shortening the multiplication time of yeast biomass. At the same time, these data suggest that high biomass yield obtained in shorter time, at comparable growth rates and generation time, could not result from larger number of cells in culture but from an increase in their weight, which can be associated with the biosynthesis of certain cellular components.

Glycerol concentration at the level of 15 % and higher inhibited the growth of the studied yeasts regardless of pH. Hence, the achieved yields of biomass were in the order of 5.4–9.6 g dm/L medium.

In summary, deproteinated potato juice water may constitute a major source of nitrogen and minerals in the *C. utilis* ATCC 9950 strains, and the addition of glycerol at the level of 5 and 10 % for the studied yeast allows achieving the most favorable molar ratio of C/N in the developed media. It creates the opportunity of simultaneous utilization of deproteinated potato juice water and glycerol in *C. utilis* ATCC 9950 yeast culture. Further research will be aimed at increasing the efficiency of biomass yield of the studied strain in media, which were considered the best.

Structure of cell wall of the studied yeast in experimental cultures

The cell wall of yeast is composed of a complex of covalently bound polysaccharides, which includes β-glucan and α-mannan present in combination with proteins forming mannoproteins. The wall consists of approximately 10–30 % of dry matter of yeast biomass, and this contribution is dependent on the species, yeast strain, and growth conditions as well [3, 17, 30]. This range may be even increased up to 50 % [28]. It results from changes in the structure of the cell wall of the yeast and thus its thickness in response to stress stimuli from the extracellular environment [17, 42]. The thickness of the yeast cell wall is ranged between 60 and 200 nm on average, depending on the

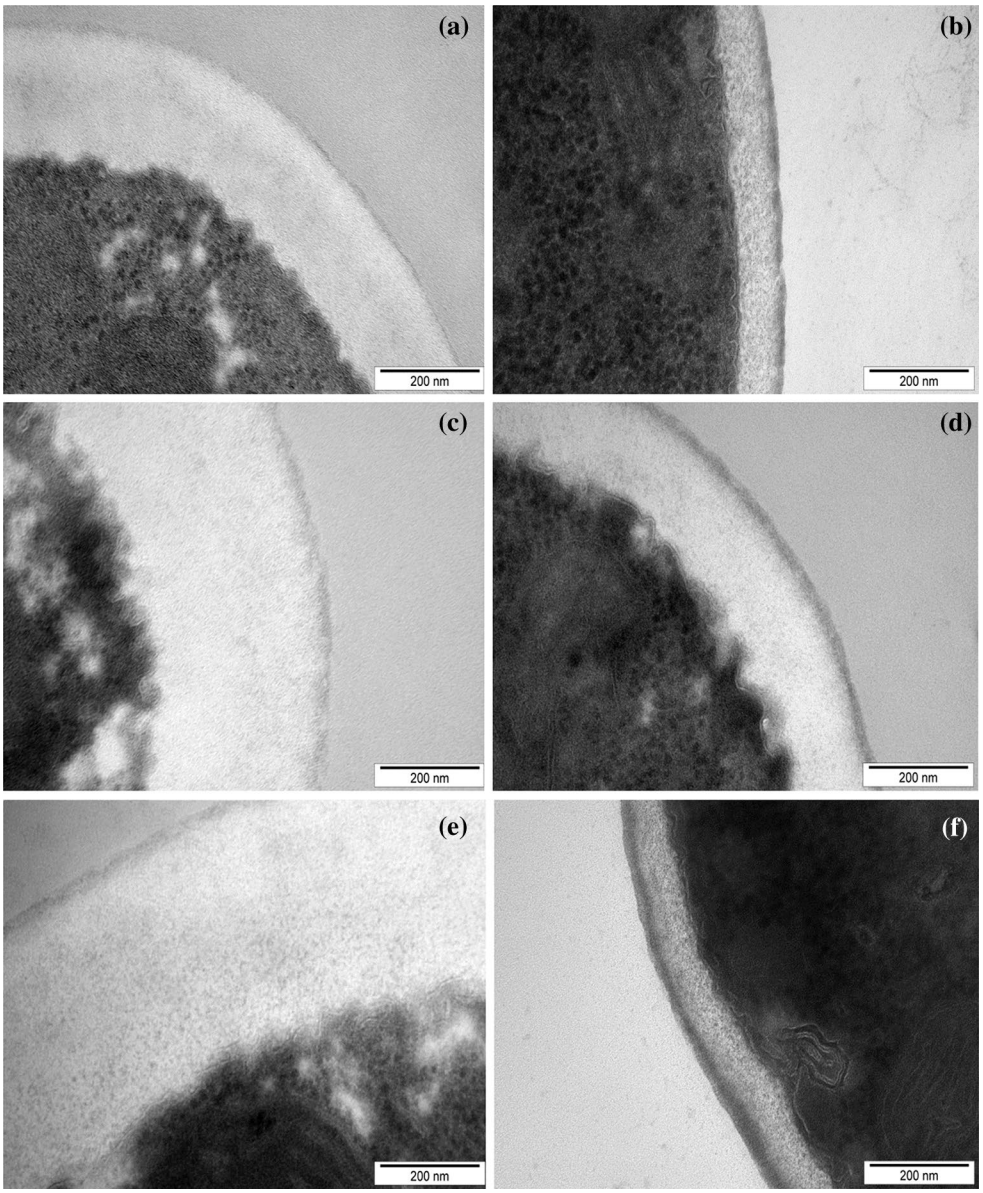

Fig. 2 Exemplary microscopic (TEM) photographs of the yeast *C. utilis* ATCC 9950 strain cell walls from experimental media. **a** YPD, **b** pH 5_5 %, **c** pH 5_10 %, **d** pH 4_25 %, **e** pH 5_25 %, and **f** pH 7_25 %

above-mentioned factors [3, 16]. TEM confirms the existence of two structural layers in the wall of yeasts: outer mannoprotein layer and inner, more transparent β-glucan layer [3].

The analysis of the structure of cell wall in the studied yeast *C. utilis* ATCC 9950 strain in experimental cultures was based on the measurement of width of the organelle and its structural layers (β(1,3)/(1,6)-glucans and mannoprotein) using TEM. Examples of microscopic images of preparations of the studied yeast cell wall in the experimental conditions are shown in Fig. 2, and the results of particular measurements are shown in Figs. 3, 4, and 5.

After culturing the studied strain in a synthetic YPD medium, an average wall thickness was approximately 188 nm (Fig. 3). The culture conditions induced changes in the content of polymers that build this organelle and thus the thickness of the wall.

Intensity and direction of changes were proved to be dependent on the concentration of glycerol in the medium and pH of the medium. Significant intensification of the synthesis of cell wall polymers in studied yeast strain occurred during cultivation in media at pH 5.0 with 5 and 10 % glycerol and in medium at pH 7.0 containing 5 % glycerol. Thus, cells of the thickest walls were observed (approximately 257, 252, and 241 nm,

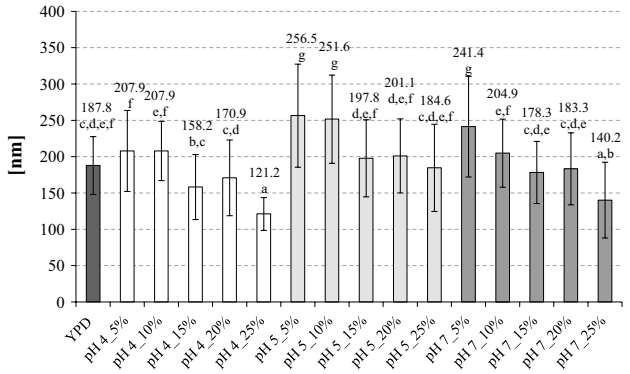

Fig. 3 Thickness of *C. utilis* ATCC 9950 strain cell wall from experimental culture (*a*, *b*, *c* mean values marked with the *same letters* do not differ significantly, Tukey's test, $\alpha = 0.05$)

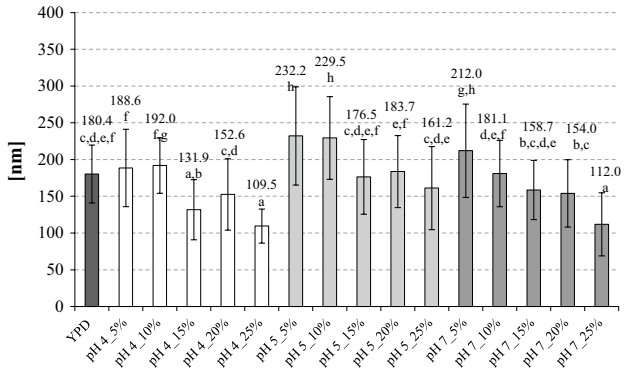

Fig. 4 Thickness of the β(1,3)/(1,6)-glucan layer in the structure of *C. utilis* ATCC 9950 cell wall from experimental culture (*a*, *b*, *c* mean values marked with the *same letters* do not differ significantly, Tukey's test, $\alpha = 0.05$)

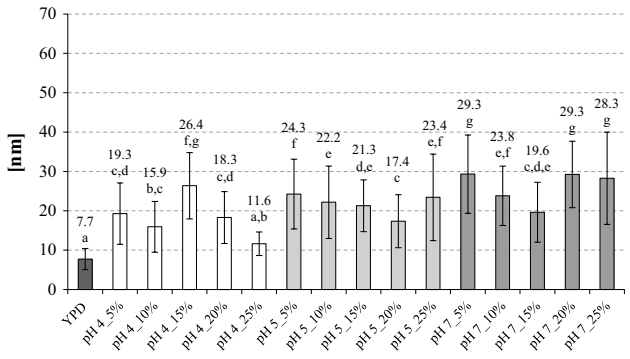

Fig. 5 Thickness of the mannoprotein layer in the structure of *C. utilis* ATCC 9950 cell wall from experimental culture (*a*, *b*, *c* mean values marked with the *same letters* do not differ significantly, Tukey's test, $\alpha = 0.05$)

respectively)—Figs. 3b, c and 4. These values were approximately 50–70 nm higher as compared with the thickness of cell walls from YPD control medium (Fig. 3a). It was observed that the addition of glycerol in concentrations higher than 15 % contributed to the reduction in the cell wall thickness of the studied yeast.

Changes in the cell wall thickness of the studied yeasts correlated with changes in thickness of the layer consisting of β(1,3)/(1,6)-glucans (Fig. 4). The most intense synthesis of these polysaccharides was found in *C. utilis* ATCC 9950 cells from media at pH 5.0 with 5 and 10 % addition of glycerol (approximately 230 nm). In *C. utilis* cells from YPD medium, the thickness of β-glucan layer was approximately 180 nm. In media with deproteinated potato juice water containing glycerol at doses above 15 %, synthesis inhibition of cell wall β-glucans of fodder yeast was observed, which was particularly revealed in media at pH 4.0 and 7.0. In these layers, the thickness of β-glucan layer was in the range from approximately 110 to 159 nm.

The process of the synthesis of yeast cell wall β(1,3)/(1,6)-glucans and its subsequent modifications occur in response to stimuli received through stress sensors of the cell wall [42]. It occurs within the framework of the so-called cell wall integrity pathway mechanism, activated by adverse changes in the environment. The yeast cells may respond to these changes in various ways, among others, by increasing the synthesis of components that build the wall [42]. This effect is mediated, inter alia, by transcriptional activation of GSC2/FKS2 genes, responsible for the synthesis of alternative Fks2p catalytic subunit of β(1,3)-glucan synthase (EC 2.4.1.34) [24, 26, 39, 42]. Simultaneously, Rho1p regulatory subunit of this protein induced by Rom2p factor exchanging GDP for GTP undergoes activation [24, 42]. In yeast cells, expression of Fks2p subunit is stimulated by low levels of glucose or its depletion in the culture medium, culturing in the presence of nonglucose-derived source of carbon such as galactose, glycerol, and acetates or other factors including increased content of Ca^{2+} ions [24]. Apart from GTP, β(1,3)-glucan synthase can be activated by other phosphorylated substances [6].

Analyzing the thickness of the mannoproteins in cell walls of studied yeasts from experimental culture (Fig. 5), one noted that media prepared with deproteinated potato juice water and glycerol intensified its synthesis in the cell wall structure.

Increased contribution of mannoproteins in the structure of cell wall was found after culturing *C. utilis* ATCC 9950 strain in media with deproteinated potato juice water at pH of 7.0 with the addition of 5, 20, and 25 % glycerol. Hence, one observed approximately four times thicker mannoprotein's layer (approximately 28–29 nm) as compared with cell walls from YPD medium (approximately 7.7 nm). Increase in the thickness of mannoprotein layer in *C. utilis* cells cultured in media with the addition of glycerol could result from the synthesis of glycoproteins necessary for cells during the metabolism of glycerol and regulation

of osmotic pressure changes in the medium caused by this carbon source. Mannoproteins constitute, among others, protection for yeast cells from osmotic stress [20].

The content of total sugars and β(1,3)/(1,6)-glucans in *C. utilis* ATCC 9950 strain cell wall preparations from experimental culture

Preparations of studied yeast cell wall were obtained by mechanical disintegration of cells. The objective of this procedure was to release the biomass of intracellular content and separate fragments of cell wall. Based on microscopic observations carried out after the disintegration of yeasts, it was found that the process was effective (Fig. 6).

Most cells underwent completed fragmentation under the action of break-shearing forces. It contributed to the effective cleaning of the walls' preparations from cytosol fragments. In aqueous environment, the occurrence of characteristic spherical aggregates of material containing fragments of studied yeast walls was reported. It confirmed the presence of hydrophobic particulated β-glucans of the wall occurring in complexes with mannoproteins. The sizes of formed aggregates were in the range from several to approximately 100 μm. Similar phenomenon was described by Hunter et al. [13] when studying the structure of cell wall structural polymers in *S. cerevisiae*.

Yeast cell wall is formed mainly by polysaccharides, and their content is determined by growth conditions [1, 14, 19]. In preparations of *C. utilis* cell walls obtained from experimental culture, one found different concentration of total sugars (Table 4).

The contribution of these components in the wall was increased after culturing the cells in media with deproteinated potato juice water at pH 5.0 with the addition of 5 and 10 % glycerol (Table 4). The content was then estimated at approximately 77–80 g/100 $g_{d.s.}$ specimen at about 55 g sugars per 100 $g_{d.s.}$ specimen of cell walls from YPD medium. Higher content of sugars in cell wall preparations proves lower content of protein and fat, which can be translated to a reduction in the costs of β(1,3)/(1,6)-glucans isolation during the formation of the purified preparations. Yeast cell wall polymers that are not purified from proteins and lipids may also be used industrially, among others, as fodder additives for animals, which act as adsorbents of toxic molds, immunostimulatory substances, or prebiotics [38, 40].

The results of total sugar content were correlated with the content of β(1,3)/(1,6)-glucans in cell wall preparations of studied yeasts (Table 4). β-Glucan biosynthesis in the walls of *C. utilis* ATCC 9950 strains was best intensified by media prepared from deproteinated potato juice water

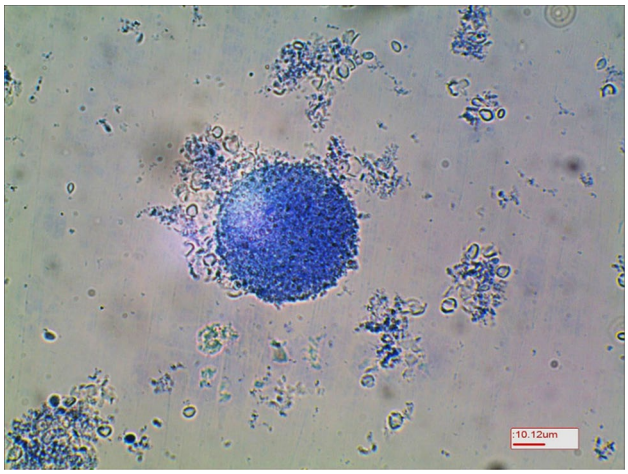

Fig. 6 Aggregates of material containing *C. utilis* ATCC 9950 strain cell wall components after the disintegration in a bead mill (magnification × 600)

at pH 5.0 with 5 and 10 % addition of glycerol. Under these conditions, the content of these polysaccharides in the walls of the studied strain was approximately 44–45 % and was significantly higher in comparison with the content after culturing in YPD medium (approximately 31 %). The studied preparations were not purified from proteins and lipids, which could possibly lower the results of the enzymatic assay used to determine β-glucans. Proteins and lipids could impede the access of polysaccharide substrate to the active center of the enzyme hydrolyzing glycosidic bond in determinations of β-glucans [14]. According to the literature [1, 14], the content β-glucans in the yeast cell wall is changeable and occurs within a wide range, representing 29–64 % of average cell wall.

Desai et al. [11] determined the productivity of β-glucans in culturing of *S. cerevisaie* baker's yeast in media with different contents of glucose, various nitrogen sources, Mg^{2+}, and Mn^{2+}. The largest amounts of the above-mentioned cell wall polysaccharides were found after culturing of yeasts in media containing 7.5–10 % glucose, 0.5 % peptone, and 0.3 % malt and yeast extract each without the addition of $MgSO_4$ and $MnSO_4$ salts. The addition of glycerol to media with deproteinated potato juice water at a dose of higher than 15 % led to a reduction in the content of β-glucans in cell walls of the studied yeasts regardless of pH and intensified the synthesis of mannoproteins. This was confirmed by microscopic observations of cell wall structure.

Due to the lack of literature data, which could be used in more thorough discussion of the obtained results and explaining the observed relationships, further research will be directed to investigate the mechanism determining an increase in the content of β(1,3)/(1,6)-glucans in the cell

Table 4 Content of total sugars and β(1,3)/(1,6)-glucans in *C. utilis* ATCC 9950 cell wall preparations from experimental culture

Medium	pH	Total sugars (g/100 $g_{d.s.}$ preparation of cell wall)	β(1,3)/(1,6)-glucan
YPD	5.0	54.5 ± 2.7a*	31.0 ± 1.5a
DPJW + glycerol: 5 %		58.0 ± 5.3a,b,c	33.6 ± 1.5b,c
10 %		64.0 ± 4.3d,e	37.1 ± 0.9d,e
15 %	4.0	69.4 ± 3.5f	37.0 ± 0.8d,e
20 %		54.5 ± 5.3a	31.3 ± 1.8a,b
25 %		57.0 ± 4.8a,b	35.4 ± 0.6c,d
DPJW + glycerol: 5 %		77.0 ± 5.1 g,h	43.6 ± 1.1 h,i
10 %		80.3 ± 4.8 h	44,9 ± 1.0i
15 %	5.0	75.5 ± 4.5 g	42.2 ± 1.0 g,h
20 %		68.9 ± 5.0f	42.4 ± 1.2 h
25 %		64.2 ± 5.1d,e	39.8 ± 1.9f,g
DPJW + glycerol: 5 %		67.8 ± 8.0e,f	39.3 ± 1.4e,f
10 %		66.7 ± 3.8e,f	39.2 ± 0.9e,f
15 %	7.0	63.8 ± 5.2d,e	39.7 ± 1.1f,g
20 %		62.4 ± 4.0c,d	35.4 ± 1.3c,d
25 %		60.2 ± 6.6b,c,d	35.4 ± 0.7c,d

* a, b, c mean values in columns marked with the same letters do not differ significantly (Tukey's test, $\alpha = 0.05$)

wall of *C. utilis* ATCC 9950 under the influence of culturing in media with deproteinated potato juice water at pH 5.0 containing 5 or 10 % glycerol.

In summary, the reported stimulation of polysaccharides' biosynthesis in the cell wall of *C. utilis* in selected media with deproteinated potato juice water and glycerol creates the possibility of favorable management of these sludge toward the production of *C. utilis* yeast biomass with a higher content of functional polysaccharides or the production of β(1,3)/(1,6)-glucan preparations.

Conclusions

Deproteinated potato juice water may constitute the primary source of nitrogen and minerals in a culture medium for *C. utilis* ATCC 9950 strains. Supplementation of DPJW with 5 and 10 % of glycerol affected the growth and biomass productivity of studied yeast most favorably. Modification of cell wall structure of the studied yeast strain was observed depending on the culture medium. Media at pH 5.0 with the addition of 5 and 10 % glycerol significantly intensified the biosynthesis of β(1,3)/(1,6)-glucans in the cell wall of fodder yeast studied. The selected media may find practical application in the utilization of environmentally burdensome deproteinated potato juice water and glycerol in the process of production of functional β(1,3)/(1,6)-glucans.

Acknowledgments This work was supported by the donation of Ministry of Science and Higher Education on research conducted by Young Scientists and Ph.D. students in 2013.

Conflict of interest None.

Compliance with Ethics Requirements This article does not contain any studies with human or animal subjects.

References

1. Aguilar-Uscanga B, François JM (2003) A study of the yeast cell wall composition and structure in response to growth conditions and mode of cultivation. Lett Appl Microbiol 37:268–274
2. Ashby RD, Nuñez A, Solaiman DKY, Foglia T (2005) Sophorolipid biosynthesis from a biodiesel co-product stream. JAOCS 82(9):625–630
3. Backhaus K, Buchwald U, Heppele N, Smitz HP, Rodicio R, Heinisch JJ (2011) Milk and sugar: regulation of cell wall synthesis in the milk yeast *Kluyveromyces lactis*. Eur J Cell Biol 90:745–750
4. Bohn JA, BeMiller JN (1995) (1 → 3)-β-D-Glucans as biological response modifiers: a review of structure-functional activity relationships. Carbohydr Polym 28:3–14
5. Bzducha-Wróbel A, Błażejak S, Tkacz K (2012) Cell wall structure of selected yeast species as a factor of magnesium binding ability. Eur Food Res Technol 235:355–366
6. Cabib E, Silverman SJ, Shaw JA, Das Gupta S, Park HM, Mullins T, Mol PC, Bowers B (1991) Carbohydrates as structural constituents of yeast cell wall septum. Pure Appl Chem 63(4):483–489
7. Chen J, Seviour R (2007) Medicinal importance of fungal β-(1 → 3), (1 → 6)-glucans. Mycol Res 111(6):635–652
8. Commission Implementing Decision of 24 November 2011 authorising the placing on the market of yeast beta-glucans as a novel food ingredient under Regulation (EC) No 258/97 of the European Parliament and of the Council
9. Da Silva GP, Mack M, Contiero J (2009) Glycerol: a promising and abundant carbon source for industrial microbiology. Biotechnol Adv 27(1):30–39
10. Dallies N, Francois J, Paquet V (1998) A new method for quantitative determination of polysaccharides in the yeast cell wall. Application to the cell wall defective mutants of *Saccharomyces cerevisiae*. Yeast 14(14):1297–1306
11. Desai KM, Vaidya BK, Singhal RS, Bhagwat SS (2005) Use of an artificial neural network in modeling yeast biomass and yield of β-glucan. Process Biochem 40:1617–1626
12. Hohmann S, Mager WH (2003) Yeast stress responses. Springer, Berlin
13. Hunter KW, Gult RA, Berner MD (2002) Preparation of microparticulate β-glucan from *Saccharomyces cerevisiae* for use in immune potentiation. Lett App Microbiol 35:267–271
14. Jaehrig SC, Rohn S, Kroh LW, Wildenauer FX, Listad F, Fleischer LG, Kurz T (2008) Antioxidative activity of (1 → 3), (1 → 6)-β-D-glucan from *Saccharomyces cerevisiae* grown on different media. LWT 41:868–877
15. Killick KA (1971) Modification of the composition and structure of the yeast cell wall by culture in the presence of sulfur amino acids. J Bacteriol 3(106):931–937

16. Klis FM, Pieternella M, Hellingwerf K, Brul S (2002) Dynamics of cell wall structure in *Saccharomyces cerevisiae*. FEMS Microbiol Rev 26:239–256
17. Klis FM, Boorsma A, De Groot PWJ (2006) Cell wall construction in *Saccharomyces cerevisiae*. Yeast 23:185–202
18. Kogan G, Kocher A (2007) Role of yeast cell wall polysaccharides in pig nutrition and health protection. Livest Sci 109:161–165
19. Kogan G, Staško A, Bauerová K, Polovka M, Šoltés L, Brezová V, Navarová J, Mihalová D (2005) Antioxidant properties of yeast (1-3)-β-glucan studied by electron paramagnetic resonance spectroscopy and its activity in the adjuvant arthritis. Carbohydr Polym 61:18–28
20. Kopecká M, Gabriel M, Nečas O, Svoboda A, Venkov PV (1991) Cell surface structures in osmotically fragile mutants of *Saccharomyces cerevisiae*. J Gen Microbiol 137:1263–1270
21. Kowalczewski P, Celka K, Białas W, Lewandowicz G (2012) Antioxidant activity of potato juice. Acta Sci Pol Technol Aliment 11(2):175–181
22. Kutera J, Czyżyk F (1997) Metody oczyszczania i utylizacji ścieków przemysłu rolnospożywczego oraz odchodów zwierzęcych z ferm i obiektów inwentarskich. IMUZ, Warszawa, pp 29–63 (In Polish)
23. Laroche C, Michaud P (2007) New development and prospective applications for β(1,3)-glucans. Recent Pat Biotechnol 1:59–73
24. Lesage G, Bussey H (2006) Cell wall assembly in *Saccharomyces cerevisiae*. Microbiol Mol Biol Rev 70(2):317–343
25. Leung MYK, Liu C, Koon JCM, Fung KP (2006) Polysaccharide biological response modifiers. Immunol Lett 105:101–114
26. Levin DE (2011) Regulation of cell wall biogenesis in *Saccharomyces cerevisiae*: the cell wall integrity signaling pathway. Genetics 189:1145–1175
27. Lipińska E (2013) Środowisko hodowlane jako czynnik determinujący cechy fenotypowe wybranych szczepów drożdży piekarskich: Rozprawy Naukowe i Monografie 435. SGGW, Warszawa (In Polish)
28. Lipke PN, Ovalle R (1998) Cell wall architecture in yeast: new structure and new challenges. J Bacteriol 180(15):3735–3740
29. Muniraj IM, Xiao L, Hu Z, Zhan Z, Shi J (2013) Microbial lipid production from potato processing wastewater using oleaginous filamentous fungi *Aspergillus oryzae*. Water Res 47:3477–3483
30. Nguyen TH, Fleet GH, Rogers PL (1998) Composition of the cell walls of several yeast species. Appl Microbiol Biotechnol 50:206–212
31. Novák M, Synytsya A, Gedeon O, Slepička P, Procházka V, Synytsya A, Blahovec J, Hejlová A, Čopíková J (2012) Yeast β(1,3), (1,6)-D-glucan films: preparation and characterization of some structural and physical properties. Carbohydr Polym 87:2496–2504
32. Nowak J, Górna B, Nowak W (2013) Applying filamentous fungi to biodegradation of wastewater from potato industry with simultaneous production of mould biomass for forage. ŻYWNOŚĆ Nauka Technologia Jakość 6(91):91–203 (In Polish)
33. Nowak J, Lasik M (2009) High temperature bioremediation of potato industry wastewaters by bacteria mixed culture. Nauka Przyroda Technologie 3(4):1–10 (In Polish)
34. Pęksa A, Miedzianka J (2014) Amino acid composition of enzymatically hydrolysed potato protein preparations. Czech J Food Sci 32(3):265–272
35. Pęksa A, Rytel E, Tajner-Czoper A, Kita A, Pytlarz-Kozicka M, Miedzianka J (2012) The comparison of aminoacid composition of the protein in preparations obtained from potato tubers cultivated within ecological and conventional systems. Biuletyn Instytutu Hodowli i Aklimatyzacji Rośli 266:307–317 (In Polish)
36. Raimondi S, Rossi M, Leonardi A, Bianchi MM, Rinaldi T, Amaretti A (2014) Getting lipids from glycerol: new perspectives on biotechnological exploitation of *Candida freyschussii*. Microb Cell Fact 13(83):1–11
37. Rozporządzenie Ministra Środowiska z dnia 24 lipca 2006 r. w sprawie warunków, jakie należy spełnić przy wprowadzaniu ścieków do wód lub do ziemi, oraz w sprawie substancji szczególnie szkodliwych dla środowiska naturalnego
38. Šandula J, Kogan G, Kačuráková M, Machová E (1999) Microbial (1 → 3)-*β*-glucans, their preparations, physic-chemical characterization and immunomodulatory activity. Carbohydr Polym 38:247–253
39. Schomburg D, Dörte S (1996) 1,3-beta-Glucan synthase. Enzyme Handb 12:261–266
40. Shetty PH, Hald B, Jespersen L (2006) Surface binding of aflatoxin B_1 by *Saccharomyces cerevisiae* strains with potential decontaminating abilities in indigenous fermented foods. Int J Food Microbiol 113:41–46
41. Sibtain A, Fayyaz A, Abu Saeed H (2010) Production of microbial biomass protein by sequential culture fermentation of *Arachniotus* sp., and *Candida utilis*. Pak J Bot 42(2):1225–1234
42. Smits GJ, Van den Ende H, Klis FM (2001) Differential regulation of cell wall biogenesis during growth and development in yeast. Microbiology 147:781–794
43. Thanardkit P, Khunrae P, Suphantharika M, Verduyn C (2002) Glucan from spent brewer's yeast: preparation, analysis and use as a potential immunostimulant in shrimp feed. World J Microbiol Biotechnol 18:527–539
44. Tokunaka K, Ohno N, Adachi Y, Tanaka S, Tamura H, Yadomae T (2000) Immunopharmacological and immunotoxicological activities of a water-soluble (1 → 3)-beta-D-glucan, CSBG from *Candida* spp. Int J Immunipharmacol 22(5):383–394
45. Yoshida Y, Yokoi W, Ohishi K, Ito M, Naito E, Sawada H (2005) Effect of the cell wall of *Kluyveromyces marxianus* YIT 8292 on the plasma cholesterol and fecal sterol excretion in rats fed on high-cholesterol diet. Biosci Biotechnol Biochem 69:714–723